

## I. AMENDMENTS

### A. Amendments to the Specification

Please amend the specification at the indicated paragraphs, as follows:

C1  
[0001] This application is a divisional application of U.S. Serial No. 09/245,615, filed February 4, 1999 (now abandoned), which claims the benefit of priority under 35 U.S.C. § 119 of U.S. Serial No. 60/073,605, filed February 4, 1998 (now abandoned), the entire contents of each of which is incorporated herein by reference.

[0063] Suitable prokaryotic vectors include plasmids such as those capable of replication in *E. coli* (for example, pBR322, ColEI, pSC101, PACYC 184, itVX, pRSET, pBAD (Invitrogen <sup>TM</sup> Corp., Carlsbad, CA), and the like). Such plasmids are disclosed by Sambrook (cf. "Molecular Cloning: A Laboratory Manual", second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, (1989)). Bacillus plasmids include pCl94, pC221, pTI27, and the like, and are disclosed by Gryczan (In: The Molecular Biology of the Bacilli, Academic Press, NY (1982), pp. 307-329). Suitable Streptomyces plasmids include pIJ101 (Kendall et al., J. Bacteriol. 169:4177-4183, 1987), and Streptomyces bacteriophages such as φC31 (Chater et al., In: Sixth International Symposium on Actinomycetales Biology, Akademiai Kiado, Budapest, Hungary (1986), pp. 45-54). Pseudomonas plasmids are reviewed by John et al. (Rev. Infect. Dis. 8:693-704, 1986), and Izaki (Jpn. J. Bacteriol. 33:729-742, 1978).

C2  
[0064] Suitable eukaryotic plasmids include, for example, BPV, vaccinia, SV40, 2-micron circle, pcDNA3.1 pCDN3.1 (Invitrogen <sup>TM</sup> Corp.), and the like, or their derivatives. Such plasmids are well known in the art (Botstein et al., Miami Wntr. Symp. 19:265-274, 1982); Broach, In: "The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, Cell 28:203-204, 1982); Dilon et al., J. Clin. Hematol. Oncol. 10:39-48, 1980); Maniatis, In: Cell

C2  
cont

Biology: A Comprehensive Treatise, Vol. 3, Gene Sequence Expression, Academic Press, NY,  
pp. 563-608 (1980).

---

[0068] Substances suitable for detectably labeling proteins include fluorescent dyes such as fluorescein isothiocyanate (FITC), fluorescein, rhodamine, tetramethyl-rhodamine-5-(and 6)-isothiocyanate (TRITC), ~~Texas Red~~ Texas Red<sup>®</sup> dye, cyanine dyes (Cy3 and Cy5, for example), and the like; and enzymes that react with colorimetric substrates such as horseradish peroxidase.

C3

The use of fluorescent dyes is generally preferred in the practice of the invention as they can be detected at very low amounts. Furthermore, in the case where multiple antigens are reacted with a single array, each antigen can be labeled with a distinct fluorescent compound for simultaneous detection. Labeled spots on the array are detected using a fluorimeter, the presence of a signal indicating an antigen bound to a specific antibody.

---

[0088] A cDNA microarray is prepared as follows: Total mRNA is isolated from tissue (for example, nerve cells) of a variety of species representative of different classes of organisms such as Drosophila, nematode, salmon, clam, chicken, mouse, dog, goat, spider monkey, chimpanzee, human, and the like, ~~by using the FastTrac method~~ FastTrac<sup>™</sup> kit (Invitrogen<sup>™</sup> Corp.

C4

~~Stratagene, La Jolla, CA)~~ or other common methods. mRNA is also obtained from a variety of unicellular organisms such as *E. coli*, yeast, *B. subtilis*, mycoplasma and the like. Eukaryotic mRNA is enriched from total RNA using oligo(dT) cellulose (Ausubel, et al, Short Protocols in Molecular Biology, 3rd ed. 1995, pgs 4-11 - 4-12). Equivalent amounts (for example, 1 µg) of mRNA from each source are placed in a separate well of one or more 96 well microtiter plates and precipitated with cold EtOH. The precipitate is rinsed with 70% EtOH and allowed to dry.

---

C5

[0090] The array is rehydrated by suspending the slide over a dish of ddH<sub>2</sub>O (50°C) for approximately one minute. The slide is quickly (approximately 3 seconds) dried by placing it on a surface heated to 100°C (mRNA side up). The mRNA is crosslinked to the poly-L-lysine

05 cont  
coating of the slide using ultraviolet radiation using a Stratalinker™ UV device according to the manufacturer's instructions (Stratagene Stratagene® Corp.) set at 60 milliJoules.

---

06  
[0093] The slide is left in the bath for 10 - 24 hours, then the cover slip is removed and the slide rinsed in 0.2X SSC with 0.1% SDS several times. Excess wash buffer is removed by centrifugation on a microtiter plate carrier as described above. The slide is scanned using a spectrofluorometer, such as the ~~Sean Array~~ ScanArray® 3000 fluorescent scanner (General Scanning Inc., Watertown, MA). For probes labeled with Cy5, for example, fluorescence is measured at 670 nm.

---

07  
[0101] Following hand arraying of the antibodies (approximately 20-30 nanoliters per spot), the nitrocellulose, aldehyde, and polystyrene slides were immediately blocked for 1 hour with PBST and 3% milk, washed 3 times with PBST, and hybridized with 50 µl of GAMG-CY3 for 30 minutes. Surmodics slides were incubated overnight in a moist salt chamber as recommended by the manufacturer. The following day, the Surmodics slides were processed as described above. Following hybridization all of the various slides were washed 3 times in PBST, dried and scanned using a ~~Sean Array~~ ScanArray® 3000 fluorescent scanner.

---

08  
[0103] Positively charged nylon filters (Zeta Probe Membranes, BioRad Laboratories, Hercules, CA) were hand arrayed using 1 µl of anti-His, anti-V5, anti-thioredoxin (anti-Thio), anti-FOS, anti-PLC-gamma and anti-CREB antibodies (~~Invitrogen~~ Invitrogen™ Corp., Carlsbad, CA; all antibodies were approximately 1 mg/ml). Filters were blocked for 1 hour with PBST and 3% milk, washed three times with PBST, and incubated with 1 µg/ml biotinylated D1 protein for three hours at room temperature. D1 is a creatine kinase fusion protein isolated from a human fetal heart cDNA library and cloned into the pBAD-Thio-His-TOPO vector (~~Invitrogen~~ Invitrogen™ Corp., Carlsbad, CA) to create a Thioredoxin-V5-His-creatine kinase fusion

C2  
cont  
protein. D1 was biotinylated using the EZ-Link™ Sulfo-NHS-LC Biotinylation Kit (Pierce, Rockford, IL) used according to the manufacturer's instructions).

C9  
[0105] The filters were washed 5 times with PBST, dried, and developed by immersion in ECL chemiluminescent substrate (ECL - Amersham, Arlington Heights, Illinois) or the chromogenic substrate BCIP/NBT (Sigma Chemicals, St. Louis, MO). Filters developed with ECL were exposed to ~~Kodak~~ Kodak® chemiluminescent film for 1 to 10 seconds.

C10  
[0107] The experiment was repeated using an array created with an automated arrayer. Antibodies (1 mg/ml) were spotted using an automated 96 pin microarrayer developed at Invitrogen Invitrogen™ Corp. Fifteen negative control antibodies (assorted mouse monoclonals) were arrayed along with the three positive control antibodies (anti-His, anti-Thio, anti-V5). Filters were treated as described above using the alkaline phosphatase conjugate and the chromogenic substrate BCIP/NBT.

C11  
[0110] To demonstrate specific binding to polyclonal antibodies, six antibodies were arrayed by hand on a nitrocellulose slide, three polyclonal antibodies (anti-E12 (unpurified rabbit polyclonal sera to a His-V5-thioredoxin-thymidine kinase fusion protein), anti-lexA (lexA repressor protein), and anti-GFP (Green fluorescent protein)) and three monoclonal antibodies (anti-V5, anti-His and anti-GalU (a mammalian transcription factor)). The slide was blocked with PBST and 3% milk for 1 hour at room temperature, and incubated with the E12-biotin conjugate, prepared according to the protocol used for D1 protein. Following extensive washing with PBST, the slides were incubated with streptavidin-CY3 conjugate (Amersham, Arlington Heights, IL) for 1 hour at room temperature, washed 5 times with PBST and dried by centrifugation prior to scanning on the ~~Scan Array~~ ScanArray® 3000 fluorescent scanner.